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Medical Science

Comparative Evaluation of the Ultrastructure of the Hippocampus in Adult Male Wistar Rats Subjected to Chronic Loud Noise

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Noise pollution is a severe public health problem. Even noise levels between 55−65 dB can lead to various undesirable alterations, including cognitive changes. While such changes have been described in a large number of studies, there is insufficient information regarding the effects of noise on the structure of brain regions involved in cognition. In the present electron microscopic study, we evaluate the ultrastructure and presynaptic architecture of hippocampal CA1 area in adult male Wistar rats chronically exposed to 100 dB noise, one hour daily for 10 consecutive days. In about 10% of large neurons, ultrastructural changes were found. In the majority of such cells, moderate or mild reversible modifications were observed. However, in another part of altered neurons significant pathologies, such as different stages of apoptosis or chromatolysis, as well as swelling or destruction of some cytoplasmic organelles were detected. The analysis of different parameters of axo-dendrites synapses revealed quantitative alterations in presynaptic mitochondria, which indicate to the changes in the production of energy, needed for neurotransmission. The results indicate that even short and intermittent chronic white noise may produce detrimental consequences on fine organization of the hippocampus - brain region involved in cognition and sound processing. *© 2022 Bull. Georg. Natl. Acad. Sci.*

White noise, central nucleus of amygdala, hippocampus, electron microscopy

Chronic exposure to loud noise is a significant problem for the society. Every day people are exposed to various types of environmental noise, produced by different sources, such as transport, home appliances or recreational or industrial activities [1]. Loud noise especially affects the population of urban areas or individuals living close for military or conflict zones [2]. According to the World Health Organization, more than 20% of Europeans from urban regions are negatively affected by noise, exceeding 65 dB, which is the safety threshold [1]. Besides disorders of auditory system, harmful effects include the development of various neurological states and altered cognition [3]. However, while the alterations in auditory system have been studied extensively (among alterations are the delay of functional organization, changes in cell signaling, other molecular modifications), there is conflicting information regarding the effects of noise on cognition. Thus, some studies indicate that noise has no effect on cognition, even if the individual experiences a high level of discomfort, stress or annoyance [4], while others show that depending on factors (type of noise, timing of exposure, the age of the individual) and cognitive task, noise may affect the cognition [5]. The majority of such data are gained from behavioral studies, while the structure of cognitive brain is described in a few studies. In addition, there are only single data regarding ultrastructural effect of noise in this part of brain.

In the present research, we elucidate the ultrastructure and presynaptic architecture of hippocampal CA1 area in male adult Wistar rats subjected to chronic loud noise. Besides cognition, the hippocampus is known to be involved in in sound processing.

Material and Methods

Eight adult male Wistar rats (P130-140) were used. The animals were kept in wire-meshed cage, two per cage. The environment was well-controlled, standard food pellets and water were ad libitum. The procedure of noise exposure has been described in our previous studies [6]. Briefly, four animals were subjected to 100 dB noise for ten consecutive days (1 h per day). For this purpose, two Paradigm Signature S1 P- Be loudspeakers were used. Two speakers were mounted 55 cm above the floor of the cages. Each speaker affected one cages. Sound level was constantly monitored using the microphone, suspended in a line 45 cm above the cage. The brain for electron microscopy was taken on the next day after the last noise session. Four control animals were kept without the noise exposure. All procedures were performed according guidelines proposed by the European Council. Committee of Ethics at ilia State University approved the research protocols.

Electron Microscopic Analysis

The material was prepared according conventional techniques [7]. Briefly, after pentobarbital injection (100 mg/kg, i.p.), the animals underwent transcardiac perfusion with ice-cold heparinized 0.9% NaCl, followed by 500 mL of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, perfusion pressure of 120 mm Hg. The left hemispheric tissue blocks containing hippocampal CA1 area were cut into 400 μ thick coronal slices and post-fixed in 1% osmium tetroxide for 2 h. The region of interest, under optical microscope Leica MM AF, was cut out from slices, dehydrated and embedded in araldite. 70–75 nm thick sections were prepared, placed on 200-mesh copper grids, stained with uranyl-acetate and lead-citrate, and examined with JEM 1400. From each rat, every fifth section, totaling 20 sections was evaluated.

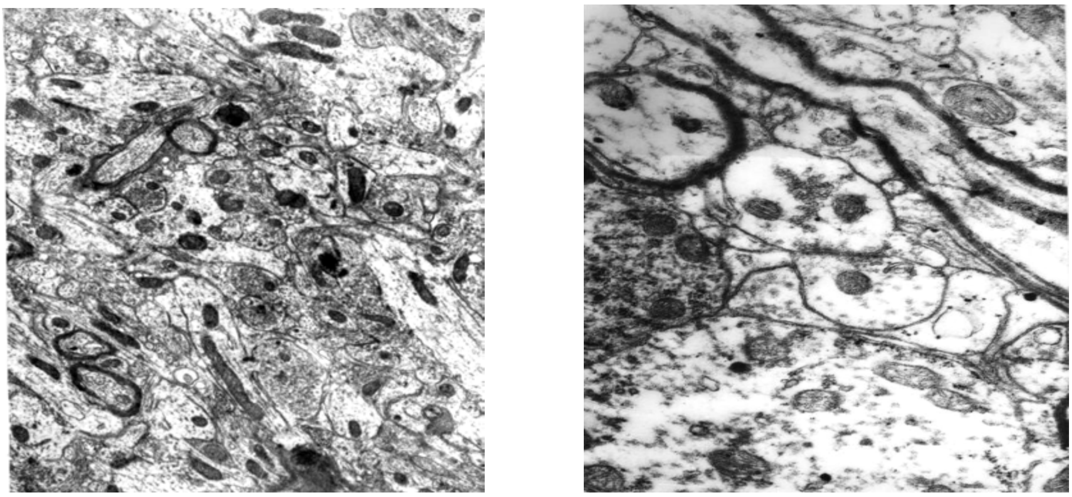
Quantitative EM study. On 120-120 presynaptic terminals from experimental and control brains (30 sections from each rat), area of presynaptic terminals, number of presynaptic mitochondria and their area were measured (ImageJ, version 1.44). To reveal possible effect of noise on these parameters, two-sample t-test was used. p-value<0.05 was considered as significant.

Results

About 10% of large neurons were changed. In approximately 5% of them, mild or moderate alterations of organelles were detected. In others, the changes were more prominent. Thus, various stages of apoptosis, peripheral or partial chromatolysis, swelling or destruction of mitochondria, enlarged cisterns of endoplasmic reticulum and Golgi body or membrane-like inclusions were detected. Some large dendrites were swelled; few axons were partially demyelinated. Several presynaptic terminals contained only few or agglutinated synaptic vesicles, single preterminals were fully degenerated (Fig. A and B). A number of astrocytes, including peri-capillary forms, were activated or contained single destructed organelles. In a few cases, dark processes, resembling dark microglia were detected. In some "normal" cells, nuclear invaginations, and concentrations of organelles and lysosomes were noticed. Quantitative analysis did not reveal between-group difference between the area of axon terminals (control = 5,739,327±1,159,054; experiment = 8,051,116±1590790. P-Value=0.246) and the number of presynaptic mitochondria (control=6.20±1.0, experiment=8.40±1.2. P-Value=0.171) (Fig. 3A, B). But in noise-exposed rats significant increase of mitochondrial area was detected (control = 279,158±61359, experiment = 1,799,645±235364. P-Value=0.000).

Discussion

According to the present results, even relatively short and intermittent chronic noise alters the ultrastructure of hippocampal CA1area. Besides its key role in cognitive processes, the hippocampus is actively involved in sound processing [8, 9]. Earlier we show the effect of chronic loud noise on the ultrastructure of porosome and fine architecture of two subcortical auditory regions: colliculus inferior and medial geniculate body [10]. In both areas, we detected alterations in porosome and irreversible pathologies in a number of neurons. Such alterations, which were confined to the changes in porosome central plug, indicate to possible changes in neuronal plasticity and transmission. Here we show that in addition to auditory areas, loud noise affects fine structure of brain region, involved in cognition. Such data coincide with a number of behavioral studies, showing that loud noise alters cognition [5,11,12]. But in noise-exposed rats we also detected a number of neurons with nuclear membrane invaginations, concentrations of organelles and lysosomes and activated glia. It remains to determine whether such modifications



A B

Fig. Neuropil of hippocampal CA1 area of adult male Wistar rat subjected to chronic noise exposition. A – In neuropil fully degenerated axon terminals are seen. B – Presynaptic terminal with agglutinated synaptic vesicles; close to highly osmiophilic active zone only few synaptic vesicles were observed.

contribute to the insult or have compensatory character. Of special interest are the dark processes in noise-exposed brain, which could represent dark microglia – the most active form implicated in conditions, related to stress [13]. The nature of such changes needs further clarification.

In our study, we analyzed presynaptic mitochondria, which provide energy for neurotransmission. The phenotypes of such mitochondria vary depending on various factors, including synapse functioning [14]. Therefore, such analysis may provide valuable information regarding transmission state. We detected significant increase of presynaptic mitochondrial area, which should reflect the increase of mitochondrial function and elevated synaptic activity. Notably, mitochondria in presynaptic terminals with huge synaptic activity optimized the ultrastructure for high rate metabolism as these presynaptic areas contain higher levels of respiratory chain proteins compared to mitochondria in presynaptic profiles with lower functional activity [15]. In this regard, it is notable that in auditory brain chronic loud noise induces sustainable increase of metabolic activity in a time-dependent way, which may lead to hearing loss; however, further research of this phenomenon is needed. It is notable that the effects of artificial loud noise are distinct from the sounds of nature. Thus, the sounds of waterfall produce healing effects in a person [16]. Comparative study of such effects should be of special importance.

Conclusion

The results show, that even relatively short and intermittent chronic noise alters the ultrastructure of the hippocampus, region, actively involved in cognition and sound processing.

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#აზერბაიჯანის სამედიცინო უნივერსიტეტი, ჰისტოლოგიის, ციტოლოგიის და ემბრიოლოგიის დეპარტამენტი, ბაქო, აზერბაიჯანი

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ხმაურით გარემოს დაბინძურება საზოგადოებრივი ჯანდაცვის უმნიშვნელოვანესი პრობლემაა. ისეთი ხმაურიც კი, რომელიც 55-65 დბ სიხშირის ფარგლებში მერყეობს, ორგანიზმში მთელ რიგ არასასურველ ალტერაციებთან, მათ შორის, კოგნიტური ცვლილებების განვითარებასთან ასოცირდება. ასეთი ცვლილებები მთელ რიგ კვლევებშია აღწერილი, თუმცა მწირია ინფორმაცია იმის თაობაზე, თუ როგორ ისახება ასეთი ზემოქმედება შემეცნებით პროცესებში ჩართული თავის ტვინის უბნების აღნაგობაზე. წარმოდგენილ ელექტრონულ-მიკროსკოპულ კვლევაში შესწავლილია ჰიპოკამპის CA1 ველის ულტრასტრუქტურა და პრესინაფსური აღნაგობა ზრდასრულ მამრ ვირთაგვებში, რომლებიც 10 დღის განმავლობაში, დღეში 1 საათი, 100 დბ ხმაურის ზემოქმედების ქვეშ იმყოფებოდნენ. ჰიპოკამპის დიდი ზომის ნეირონების დაახლოებით 10%-ში აღწერილ იქნა ულტრა-სტრუქტურული ცვლილებები. მათი დიდი ნაწილი მეტწილად მსუბუქი, ზომიერი ან შექცევადია. ამასთანავე, შეცვლილი ნეირონების მცირე ნაწილში გამოვლინდა მნიშვნელოვანი პათოლოგიები, როგორიცაა აპოპტოზის ან ქრომატოლიზის სხვადასხვა სტადიები, და ზოგიერთი ციტოპლაზმური ორგანელების გაჯირჯვება ან დესტრუქცია. გარდა ამისა, აქსო-დენდრიტული სინაფსების სხვადასხვა პარამეტრების მორფომეტრიული ანალიზით გამოვლინდა პრესინაფსური მიტოქონდრიების რაოდენობრივი ცვლილებები, რაც მიუთითებს, რომ რიგ პრეტერმინალებში ნეიროტრანსმისიისთვის საჭირო ენერგიის პროდუქცია ირღვევა. ამგვარად, ჩვენი მონაცემების თანახმად, თუნდაც შედარებით ხანმოკლე მაღალი სიხშირის ქრონიკული თეთრი ხმაური უარყოფითად მოქმედებს ჰიპოკამპის ნატიფ ორგანიზაციაზე – თავის ტვინის უბანზე, რომელიც კოგნიტურ ფუნქციებსა და სმენითი ინფორმაციის გადამუშავებაში უმნიშვნელოვანეს როლს ასრულებს.

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